18. (New) The composition according to claims 17, for the treatment of tumours.

D1

19. (New) The composition according to claim 17, for the treatment of diseases caused by bacteria, fungi, protozoa or viruses, in which said bacteria, fungi, protozoa or viruses show the capacity to bind the long pentraxin PTX3.--

REMARKS

Reconsideration is requested.

Claims 1-8 and 12-16 have been canceled, without prejudice.

Claims 17-19 have been added, and upon entry of the present Amendment, will be pending.

Attached are a copy of corrected drawings however the same are not believed to be required as, by the Examiner's own "information" provided on page 3 of the Office Action dated May 20, 2002 (Paper No. 17), correction of informalities are not required until, at the latest, three months after the issuance of a Notice of Allowability. As the corrections required in the Form PTO-948 are merely corrections relating to formal issues, corrections should not be presently required to avoid abandonment, as stated by the Examiner on page 3 of Paper No. 17. In any event, to advance prosecution, formal drawings are attached. Nothing further should be required in this regard.

The Section 112, second paragraph, rejection of claims 1-5 is obviated by the above amendments. The objections noted to claim 1 on page 4 of Paper No. 17 have been corrected in the newly presented claim 17. Accordingly, entry of the above

amendments will, at a minimum, reduce the issues for appeal by obviating the Section 112, second paragraph, rejection of claims 1-5.

Entry of the amendments is requested.

The Section 112, first paragraph, rejection of claims 1-5 noted in paragraph 5 of Paper No. 17 will be moot upon entry of the present Amendment. The newly presented claim 17 is supported by enabling disclosure, as noted by the Examiner. See, page 5 of Paper No. 17 ("while being enabling for a pharmaceutical composition comprising a single human PTX3 protein (i.e. SEQ ID NO: 1)..."). Entry of the above amendments will, at a minimum, reduce the issues for appeal by obviating the Section 112, first paragraph, rejection of claims 1-5 noted in paragraph 5 of Paper No. 17. The claims have been amended to advance prosecution and without prejudice. Entry of the above amendments is requested.

The applicants note in response to the Examiner's comments in the first paragraph of page 6 of Paper No. 17 that the amended claims are directed to compositions for treating diseases caused by bacteria, fungi, protozoa or viruses which show the capacity to bind to PTX3. The Examiner is requested to consider the following experimental data in this regard.

PTX3 is capable to bind pathogens

In Table 1 are reported some examples of pathogen agents which are above to bind PTX3.

TABLE 1

Pathogen	**Binding**

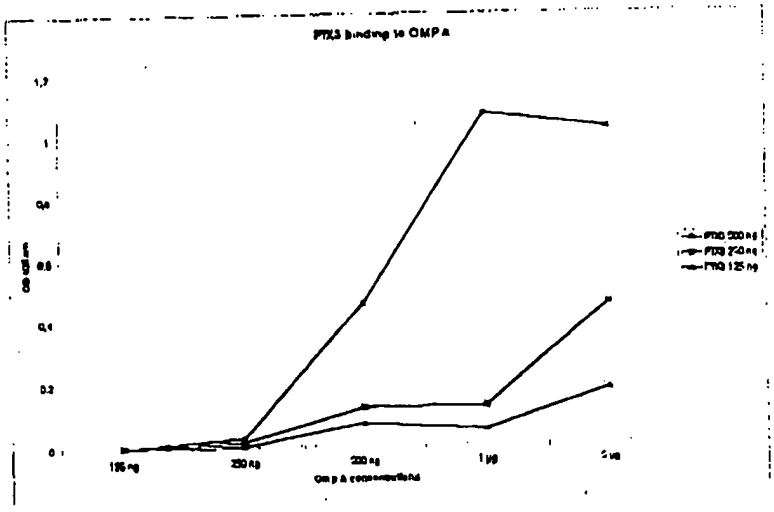
Aspergillus fumigatus	+
Pseudomonas Aeruginosa	+
Salmonella Tiphymurium	+
Staphylococcus Aureus	+

Legend: The indicated pathogens were incubated with a biotin labeled PTX3 (20µg/ml) and analyzed b FACS with Streptavid-FITC. (+) indicate that PTX3 treated cells showed a significant increase of the mean fluorescence intensity (MFI) with respect to PTX3 untreated cells.

PTX3 is capable of binding all Gram positive bacteria

Figure 1 shows that **PTX3** is able to **bind** in vitro the outer membrane protein A (Omp A) which is expressed on the membrane surface of **all** the **Gram positive** bacteria.

Figure 1

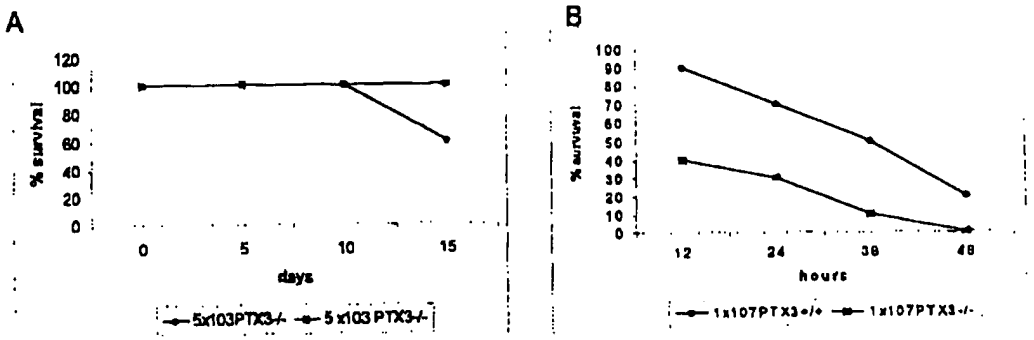


Legend: The indicated concentrations of Omp A have been absorbed on 96 multiwell plate.
Biotin labeled PTX3 at the indicated concentrations has been incubated on Omp A coated plate.
Streptavidin-HRP has been used to measure the relative amount of PTX3 bound to Omp A.

Role of PTX 3 in Salmonella typhimurium and Pseudomonas aeruginosa infection

To assess the role of PTX3 in protecting from Salmonella typhimurium and Pseudomonas aeruginosa infection, PTX3 -/- mice has been injected with the above mentioned bacteria and analyzed for survival in comparison with PTX3 +/+ mice.

Figure 2



Legend: A) The indicated doses of Salmonella Typhimurim were injected intraperitoneally in both $PTX3^{+}/^{+}$ and $PTX3^{-}/^{-}$ mice (n=8). Mortality was daily monitored. B) The indicated doses of Pseudomonas Aeruginosa were injected i.t. (intratracheally) in both $PTX3^{+}/^{+}$ and $PTX3^{-}/^{-}$ mice (n=8). Mortality was monitored every 12 hours.

The results reported in Figure 2 show that $PTX3^{-}/^{-}$ mice are more susceptible to Salmonella typhimurium and Pseudomonas aeruginosa infection then $PTX3^{+}/^{+}$ mice in terms of MST and mortality. This is a clear demonstration that PTX3 interaction with bacteria is required to protect against pathogens.

Role of PTX 3 in resistance to invasive pulmonary aspergillosis

PTX3 bound *Aspergillus fumigatus* conidia *in vitro*, this suggests a protective role for PTX3 in a murine model of invasive pulmonary aspergillosis.

PTX3 $^{-/-}$ and $^{+/+}$ mice were challenged with 2 x 10^8 spores of *A. fumigatus* intratracheally. Mice were monitored for mortality, fungal load and pathology in the organs. As shown in Table 2, wild type mice survive to *A. fumigatus* in this model of infection. In contrast, in two different experiments performed, PTX 3 $^{-/-}$ mice showed a MST of 3 days and a survival rate of 0%. *A. fumigatus* invasiveness was also assessed as fungal burden in lungs and brain. As shown in Table 2 the increased susceptibility of PTX3$^{-/-}$ mice correlated with a dramatic increase in lung colonization at day three of infection, with a 1000-fold increase in lung CFU in PTX3 $^{-/-}$ mice. The brain was not colonized in wild type mice, while in PTX 3$^{-/-}$ mice fungal burden in the brain was high (10^5 - 2 x 10^5 CFU/brain).

Mortality rate, MST and fungal burden in PTX3 $^{-/-}$ were equivalent to or worse than those obtained in PTX3 $^{+/+}$ mice after depletion of polymorphonuclear cells by treatment with anti-Gr-1 (RB6-8C5) (Table 2).

In two *in vivo* experiments PTX3$^{-/-}$ mice were treated with 20µg of purified hPTX3 intratracheally at the time of challenge (day 0) and intravenously (day 1 and 2). As shown in Table 2 the phenotype was reverted and treated PTX3$^{-/-}$ mice behaved as PTX3$^{+/+}$ mice: mortality rate was reverted to 0/4 and MST was more than 60 days as in PTX $^{+/+}$ mice. Lung burden was reduced 4-fold by treatment. **The restoration of resistance to invasive pulmonary aspergillosis (IPA) in PTX3 $^{-/-}$ mice by PTX3 administration confirms the critical and specific role of PTX3 in this fungal infection.**

TABLE. 2- Susceptibility of PTX3 -/- mice to invasive pulmonary *aspergillosis*

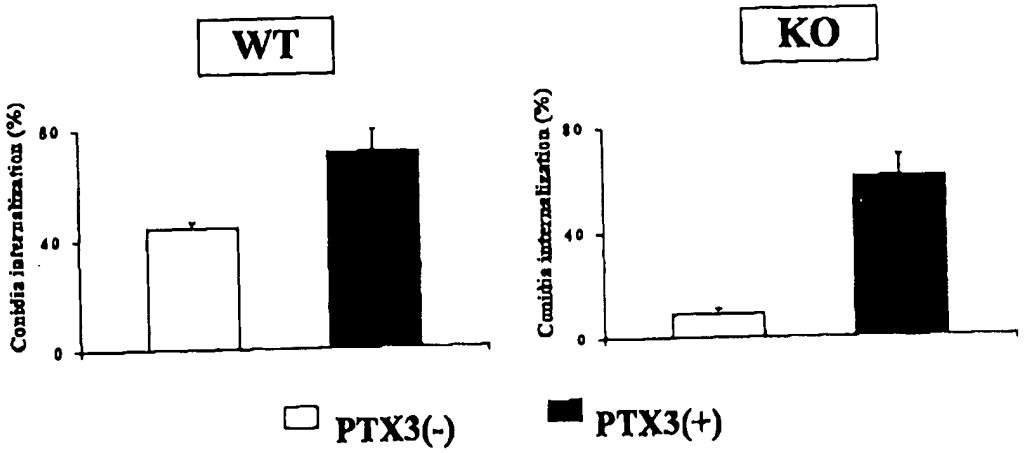
Mice	Treatment (a)	MST (days) (b)	Dead/total	Brain CFU (c)	Lung CFU (c)
Exp. 1					
PTX3 +/+	None	>60	0/3	0	8100
PTX3 +/+	RB6-8C5	4	4/4	34800	170100
PTX3 -/-	None	3	3/3	142200	706500
PTX3 -/-	RB6-8C5	3	3/3	187200	603750
Exp. 2					
PTX3 +/+	None	>60	0/6	ND	12900
PTX3 -/-	None	3	7/7	ND	233250
PTX3 -/-	PTX3*	>60	0/4	ND	60900

Legend: Mice were infected intratracheally with *A fumigatus* conidia (2 x 10^8/mouse) on day 0. (a) Mice were treated with RB6-8C5 monoclonal antibody (100 µg/mouse) intraperitoneally 2 h before fungal challenge to obtain PMN depletion or with(*) 20 µg PTX3 intratracheally on day 0 and intravenously on day 1 and 2. (b) MST: median survival time. (c) CFU were determined at day 3 after infection.

PTX3 improve Phagocytosis of A. fumigatus conidia by alveolar macrophages an in vitro internalization assay.

The ability of alveolar macrophases to ingest resting conidia in vitro, was significantly impaired in PTX3 $^{-/-}$ mice, as compared to PTX3 $^{+/+}$ mice (Fig. 3). However, PTX3 restored the phagocytic activities of cells from PTX3 $^{-/-}$ mice and potentiated PTX3 $^{+/+}$ mice (Fig. 3) This phagocytosis assay on PTX$^{+/+}$ macrophases is a further indication of the therapeutic activity of PTX3 in pulmonary infections.

Figure 3



Legend: Alveolar macrophases isolated from the indicated mice (2 x 10^5 cells/200 µl) obtained by plastic adherence from the bronchoalveolar lavage fluid, were incubated at 37 C for 2h with 10^6 conidia in 6 ml polypropylene tubes (N. 2063, Falcon), in 200 µl of Iscove medium containing 5 µg/ml polymixin B (Sigma) and 50 µl/ml gentamycin but no FCS to avoid non specific activation by serum components. Phagocytic cells were separated from non phagocytosed *A. fumigatus* cells by centrifugation on a fetal serum gradient. Harvest phagocytic cells was used for cytospin preparation. After Diff Quik staining fungal cell internalization was express according to the following formula: Conidia internalization = number of cell containing one or more fungal cells / 100 cells: In PTX3 (+), 20 µg/ml PTX 3 was added.

Therapeutic function of PTX3 in a murine T-cell depleted model of invasive pulmonary asperigillosis

Asperigillus fumigatus is a major opportunistic pathogen in immunodeficient patients and poses a formidable therapeutic challenge. The applicants investigated whether administration of PTX3 was active in an invasive pulmonary aspergillosis model of allogeneic, T-cell depleted, bone marrow transplantation (BMT) in $PTX3^{+}/^{+}$ mice. As shown in Table 3, combined systemic and local PTX3 administration caused a significant two-fold increase in survival time with two out of eight mice being cured.

Moreover, the lung CFU counts were drastically reduced (> four-fold) in PTX3-treated mice.

Table 3

Mice	Treatment	MST (days)	Dead/total	Brain CFU	Lung CFU
BMT	None	3	8/8	ND	814310
BMT	PTX3*	8^{+}	6/8	ND	187300^{+}

Legend: Mice underwent allogeneic T-cell-depleted BMT as described in Mencacci, A. et al. *Blood* **97**, 1483-90. (2001) were infected intratracheally (i.t.) with *A fumigatus* conidia ($2x10^8$/mouse) 7 days later. PTX3 was given on day 0 i.t. and on day 1 and 2 i.v. (*, 20 µg/mouse). $^{+}p<0.05$ compared to control mice (Mann Whitney U test).

As above mentioned:

1) PTX3 binds selected microbial agents, comprising conidia of *Asperigillus fumigatus, Pseudomonas aeruginosa, Salmonella tiphimurium, Staphylococcus aureus,* and all *Gram positive* bacteria;

2) PTX3$^{-/-}$ mice show higher mortality and reduction of the medial survival time when infected with pathogens;

3) PTX$^{-/-}$ mice infected with pathogens mentioned above show lower mortality and higher medial survival time when treated with PTX3;

4) susceptibility to *A fumigatus* infection of $^{-/-}$ mice was associated with defective recognition of conidia by alveolar macrophages and indicate that conidia opsonization by PTX3 direct binding is required to reverse defective phagocytosis; and

5) pulmonary aspergillosis infection, in a mouse model of allogeneic, T cells depleted, bone marrow transplantation can be prevented by PTX treatment thus indicating the therapeutic role of PTX3 also in $PTX3^{+}/^{+}$ immune compromised mice.

While the applicants do not believe it necessary to indicate or explain a mechanism of action, and without wishing to be bound by any such action, the applicants believe that the data presented indicate that PTX3 works as soluble pattern recognition receptor (PRR) and is useful as protective agent in pathogens infection. In particular, PTX3 appears to be effective when bound to the pathogen agent.

This mechanism of action can be extended not only to all fungi or bacteria which are capable of binding PTX3, but also to protozoa or viruses that show the same capability to bind PTX3.

Accordingly, in view of the above, the applicants submit that the claimed invention is supported by an enabling disclosure. Entry of the above is requested.

To the extent not obviated by the above, the Section 112, first paragraph, rejection of claims 1-5 stated in paragraph 6 of Paper No. 17 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following.

Examiner states that anti-tumor activity by cloning human PTX3 into a murine mastocytoma p815 cell line cannot provide support for the treatment of genus tumors. See, page 6 of Paper No. 17.

On page 9 to page 10 line 3 of the text of the present application however, are reported data about the anticancer activity of the compound according to the present invention: "**Anticancer activity:** a line of murine mastocytoma **P815** was transfected

by electroporation with the expression vector pSG5 containing the cDNA of human PTX3 or its antisense.

Male DBA/2N CrlBR mice aged 8-10 weeks were subcutaneously injected with $1x10^5$ cells of P815 PTX3-producing clones or with clones containing the antisense gene. **The mice were monitored 3 times daily for occurrence of tumours and once daily for survival.**

The results obtained are reported in Table 2 and show the efficacy of PTX3, in this experimental model of gene therapy, in bringing about healing of the animals and complete rejection of the tumour after the take of the inoculated tumour cells.

These results are statistically significant with p<9,91 (Fisher test) both as compared to controls and to the group treated with the antisense" (Emphasis added).

On page 11, Table 2 of the text are reported the data of the antitumoral activity of the compound according to the present invention.

"TABLE 2	IN VIVO ANTICANCER ACTIVITY OF PTX3
Clone[1]	Reject[2]
Parent **P815** (control)	4/25
P815-AS1 (antisense)	3/8
P815-PTX3-1 (sense)	**14/14***

1: 1 x 10^5 cells of the clone indicated were injected subcutaneously.
2: Number of animals that definitely reject the tumour out of total number of animals in which it took.
*** : p<0.01 as compared both to mice treated with parent cells and to mice treated with cells of the antisense clones (Fisher test)"** (Emphasis added).

The applicants submit that the above-described aspects of the specification should be sufficient to demonstrate the antitumor activity of PTX3.

To further characterize the antitumor activity of PTX3 however, the murine melanoma cell line B16 was stably transfected with the palsmid vector pSG5hPTX3 encoding for human PTX3.

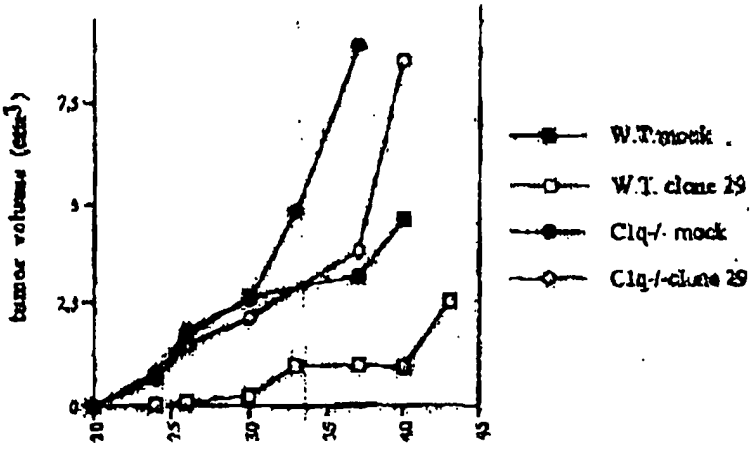
The B16 cell clone expressing the hPTX3 (PTX 29) was injected subcutaneously either in C57 mice or in C1qKO.

Untransfected B16 cells were used as control in both these mouse strains.

In the C57 mice, PTX3 transfected B16 cells showed **a significant** delay in tumor growth rate compared to untransfected parental cells (see Figure 4)

The observed delay of tumor growth rate of PTX transfected B16 cells was C1q dependant as C1q KO mice developed PTX 3 transfected and parental tumor at the same extent (see Figure 4).

Figure 4



Legend:
W.T. mock : C57/b6 mice treated subcutaneously with $1x10^5$ B16 cells;

W.T. clone 29: C57/b6 mice treated subcutaneously with $1x10^5$ B16 cells transfected with pSC5hPTX3;
C1q $^{-/-}$ mock: C1q KO mice treated subcutaneously with $1x10^5$ B16 cells;
C1q $^{-/-}$ clone 29: C1q KO mice treated subcutaneously with $1x10^5$ B16 cells transfected with pSG5hPTX3.

The following main features characterize PTX3:

1) PTX3 is able to form a decamer by the establishment of disulfides bounds among its monomers,
2) The decamer of PTX3 is able to bind the first element of the complement classical pathway C1q (Bottazzi et al. 1997).

The experiment shown in Figure 4 highlight the antitumor activity of PTX3 even in the context of the B16 melanoma cell line and indicate that decamerization and C1q binding capacity of PTX3 is required for its antitumor activity.

The data reported in the application as filed and these above further presented data are a clear demonstration and confirmation of the antitumoral activity of the compound according to the invention.

In view of the above therefore the applicants believe the specification adequately describes the presently claimed invention.

The Section 102 rejection of claims 1-5 over Alles (Blood, 1994, 84(10:8483-8493)) will be moot on entry of the above amendments. Claims 1-5 as well as the above amended claims are submitted to be patentable over the cited art and consideration of the following in this regard is requested.

The Examiner is urged to appreciate that in the section titled "Production of polyclonal antiserum" beginning on page 3484 Alles explains on page 3485, first column, lines 8-13 that "The solubilized proteins were separated in a 10%

polyacrylamide gel under reducing conditions. The gel slice containing recombinant PTX3 was excised, mechanically disrupted in saline, and injected SC into a 28-day-old rabbit (Charles River, Calco, Italy). Boosts were administered at 2, 4 and 9 weeks and serum was collected 7 days after the last injection" (Emphasis added).

The Examiner will appreciate that a pharmaceutical composition is a mixture composed of an active ingredient and at least a pharmaceutically acceptable excipient. "Pharmaeutical acceptable excipient(s)" are an excipient or a mixture of excipients which do not give rise to unwanted toxic side effects.

A composition comprising an active ingredient in admixture with a toxic excipient cannot be considered a pharmaceutical composition.

The composition mentioned by Alles is composed of PTX3, **polyacrylamide**, and saline.

The abstract of Zhonghua Zheng Xing Wai Ke Za Zhi 2002, Mar; 18(2):79-80, (copy attached), relates to an experimental study on the toxic effects of hydrophilic **polyacrylamide** gel.

In this abstract is reported: "*To study the safety of Hydrophilic* **polyacrylamide gel (HPAG)** RESULTS: It was determined that the cytotoxicity was over level-two. **The toxicity to kidney was obvious.** ...CONCLUSION: **HPAG has obvious cytotoxicity**..."" (Emphasis added).

The abstract of Rev. Environ. Health 1989 Jan-Dec; 8 (1-4) 3-16, (copy attached), relates to the Toxicity of **polyacrylamide** and acrylamide monomer.

In this abstract is reported: "... *The United States government, specifically the Food and Drug Administration and the Environmental Protection Agency, already*

regulates several uses of polyacrylamide; criteria and standards have been established based on numerous toxicological studies of both polyacrylamide and acrylamide. These studies are reviewed and summarized. **The regulations generally restrict both the amount of residual acrylamide monomer in the polyacrylamide and the amount of polymer that may be used in the specified application.** *By imposing this type of restriction, a maximum limit on the amount of acrylamide in contact with food or drinking water can be indirectly achieved"* (Emphasis added).

Therefore, if a pharmaceutical composition is a mixture composed of an active ingredient and at least a pharmaceutically acceptable excipient, a mixture comprising **polyacrylamide** is not a pharmaceutical composition and the cited art, which contains the same, can not anticipate the presently claimed invention.

The claims, as amended, are submitted in being condition for allowance and a Notice to that effect is requested.

The Examiner is requested to contact the undersigned if anything further is required in this regard.

Respectfully submitted,

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: Zhonghua Zheng Xing Wai Ke Za Zhi 2002 Mar;18(2):79-80

[Experimental study on the toxic effects of hydrophilic polyacrylamide gel]

[Article in Chinese]

Huo M, Huang J, Qi K.

Plastic Surgery Hospital, Chinese Academy of Medical Sciences, Beijing 100041, China.

OBJECTIVE: To study the safety of Hydrophilic polyacrylamide gel (HPAG) through an animal experiment. METHODS: After HPAG was injected underneath the skin of SD rats, tissue specimens were taken for general and histological examinations. The cytotoxicity was evaluated by agar coverage and MTT method. RESULTS: It was determined that the cytotoxicity was over level-two. The toxicity to kidney was obvious. The local histological reaction was slight and a thin fibrous membrane was formed around HPAG, which became stiff gradually. The shape and location of the injected HPAG was not stable. The HPAG could not be drawn out completely. CONCLUSION: HPAG has obvious cytotoxicity and is not a suitable material as soft tissue implant for the bad shape and texture.

PMID: 12192765 [PubMed - in process

1: Rev Environ Health 1989 Jan-Dec;8(1-4):3-16

Toxicity of polyacrylamide and acrylamide m nomer.

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The purpose of this paper is to present information gathered regarding, in general, the physical characteristics, and, in particular, the possible toxic nature of polyacrylamide A short discussion of the properties and toxicity of the acrylamide monomer is also included. The United States government, specifically the Food and Drug Administration and the Environmental Protection Agency, already regulates several uses of polyacrylamide; criteria and standards have been established based on numerous toxicological studies of both polyacrylamide and acrylamide. These studies are reviewed and summarized. The regulations generally restrict both the amount of residual acrylamide monomer in the polyacrylamide and the amount of polymer that may be used in the specified application. By imposing this type of restriction, a maximum limit on the amount of acrylamide in contact with food or drinking water can be indirectly achieved.

Publication Types:
- Review
- Review, Tutorial

PMID: 2485925 [PubMed - indexed for MEDLINE]